Seed Grant Application Cover Page FY2013

PRINCIPAL INVESTIGATOR:

Name:	Tanya Miura	Title:	Assistant Professor
Department:	Biological Sciences	Email:	tmiura@uidaho.edu

ADDITIONAL INVESTIGATORS:

Name:		Title:	
Department:		Email:	
Name:		Title:	
Department:		Email:	

Amount Requested: $12,000

Proposal Title: Viral Competition in Lung Epithelial Cells

PROPOSAL CHECKLIST:

- ☒ Abstract
- ☒ Narrative (2 single-spaced pages)
- ☒ Budget Page
- ☒ Biographical Data
- ☒ Publications/Exhibits/Performances (5 years)
- ☒ Proposals Submitted/Funded (5 years)
- ☐ Summary of Previous Seed Grant(s)
- ☐ Applicable animal/human requests for approval are attached

Has seed grant previously been awarded? ☐ Yes ☒ No

ELIGIBILITY:

- ☒ Early career faculty establishing scholarly program (5 years or less employment at UI)
- ☐ Established faculty transitioning into a new scholarly area

ABSTRACT

Several studies have reported the detection of multiple viruses in 10-30% of respiratory samples from young children exhibiting severe disease. Clinical studies have attempted to identify a relationship between viral co-infections and disease severity. However, their findings are not congruent, and it remains unclear how viruses interact, within the context of a host, to determine disease severity. This information is absolutely critical to design effective vaccination or therapeutic strategies for respiratory viral infections in children. To begin to address this problem, we will determine how two respiratory viruses interact within their shared target host cell: respiratory epithelial cells. Cells will be infected with 4-6 individual viruses or pairwise combinations as concurrent or sequential infections. Viral growth will be measured to determine how it is altered by co-infection. These experiments will provide an understanding of how respiratory viruses interact during co-infections, in the absence of the host's immune system. From this, we will select viruses to expand our findings into an animal model. The results of this study will be submitted for publication in the *Journal of Virology*, and used as preliminary data to support an NIH proposal to fund development of the animal model.

NARRATIVE

Acute respiratory infections are among the top five causes of death for both low and high income countries, and are a leading cause of morbidity and economic loss. Viral infections in the lower respiratory tract cause severe disease, especially in pediatric patients, and are responsible for a majority of infant hospitalizations. The development of highly sensitive diagnostic assays has increased the detection of viruses in respiratory samples, and a number of studies have reported that approximately 20% of pediatric patients with clinical respiratory disease are infected by more than one virus [1-3]. Several of these viruses, including influenza virus, respiratory syncytial virus, rhinovirus, and coronaviruses, target the epithelial cells that line the respiratory tract [4-8]. However, the interactions between these viruses within epithelial cells have not been characterized. The purpose of this study is to determine the effects of dual respiratory viral infections on viral growth dynamics in epithelial cells.

Contribution to Field and Professional Development: An understanding of how two unrelated viruses interact within respiratory epithelial cells is critical for the design of effective treatment strategies for respiratory viral infections. The ultimate goal of this research is to develop a mouse model to determine the effect of respiratory viral co-infections on disease severity and to identify the mechanisms that mediate enhanced and/or reduced disease severity compared to single virus infections. This model is critical because clinical studies cannot control the dose, timing, and strains of viral co-infections, or draw conclusions about intracellular competition between viruses or their interactions within the host's immune system. This project will be competitive for NIH funding, but requires preliminary data prior to submission. My lab does not have funding to initiate these studies, and seed grant funding would be used to perform key experiments to characterize the interactions between mouse respiratory viruses in their target cells. These data will be used to identify virus combinations in which one virus alters replication of a second virus to be used for mouse infections.

Objectives and Methods: We will infect mouse epithelial cells with 4-6 respiratory viruses individually, and in pairwise combinations of concurrent and sequential co-infections. Viral growth will be quantified by two methods. Viral plaque assay is the gold standard to measure infectious virus particles, but may not distinguish between the individual virus strains. We will also use quantitative RT-PCR (qRT-PCR), which does not distinguish between infectious and defective viral particles, but does allow us to distinguish between the individual viruses for our co-infection experiments. Data will be used to analyze the ability of each virus to alter the growth of a second virus. Murine viruses that are commonly studied as models for human respiratory viral infections will be evaluated so the findings can be used for development of a mouse model. The viruses to be used are described in the following table:

Virus:	**Human Virus Counterpart:**	**Relative Severity in Mice:**
Influenza A virus (PR8)	Seasonal influenza A virus	severe
Mouse pneumovirus (PVM)	Respiratory syncytial virus	severe
Sendai virus (SeV)	Parainfluenza virus	moderate
Mouse coronavirus (MHV-1)	Human coronavirus	moderate
Mouse adenovirus (MAV-1)	Human adenovirus	mild
Rhinovirus 1B (RV1B)	Human rhinovirus	mild

We have already shown that PR8 and MHV-1 replicate in two mouse lung epithelial cell lines, E10 and MLE15. Therefore, we will initially test these cells for susceptibility to the additional four viruses to be used in this study: PVM, RV1B, SeV, and MAV-1. Viral infection will be

confirmed by fluorescent antibody detection of viral proteins, and quantification of infectious virus in cell supernatant medium by plaque assay. Once we have identified a cell line that is susceptible to infection by all six viruses, we will establish qRT-PCR assays for the viruses based on published studies [9-11]. We may not find one mouse epithelial cell line that is universally susceptible to all six viruses. Because all of these viruses target murine respiratory epithelial cells *in vivo*, we do not expect this to be an obstacle. However, we can test other murine epithelial cell lines, for example, LA-4 cells are susceptible to infection by RV1B, PR8, and PVM. Our lab also has extensive experience in the isolation and culture of primary rodent alveolar epithelial cells [8, 12]. PR8 and MHV-1 infect the primary cultures, and these cells will be tested for susceptibility to the other four viruses if necessary. Alternatively, these studies could be carried out with fewer viral strains, focusing on those that replicate well in murine epithelial cell lines. Viral growth curves will be established for each of the individual viruses by infecting the cells at low and high doses, and quantifying production of viral genomes over time by qRT-PCR. We will then determine how the viral growth curves are affected by competition between respiratory viruses. Murine epithelial cells will be inoculated with each pairwise combination of the six viruses concurrently and sequentially. For sequential infections, the first virus will replicate for 24 h prior to inoculation of infected cells with the second virus.

Expected Outcomes and Deliverables: Viruses have strategies for maximizing their own fitness that can either inhibit or enhance infection by other viruses. Sequential virus infections are blocked by many viruses through inhibition of entry or replication of the second virus [13, 14]. In contrast, a virus can also enhance infection by a second virus by activating the cell to increase viral production or inhibiting cellular antiviral mechanisms [15-18]. Therefore, we expect to see a range of interactions, from no effect to inhibition, and even enhancement of viral replication. Some of the virus pairs may replicate independently within the epithelial cells, in which case we expect their growth curves to be the same as during single virus infections. In the case of viruses that require the same cellular resources or machinery, we expect to see competition between the viruses, and the second virus or both will have reduced viral growth. Finally, we may see enhanced replication of the second virus, if the first virus alters the cells in a way that makes them more susceptible to the second virus. These data will be used to identify virus pairs for future mouse infection studies to determine the effect of viral co-infections on disease severity within the context of the immune system of the host. We are confident that these studies will be competitive for NIH funding, but will require the proposed cell culture experiments to support an NIH proposal. The proposed study will also be submitted to the *Journal of Virology* for publication.

Distinction From Currently Funded Work: I currently have seed grant funding from the UI Host Pathogen Interaction COBRE (NIH) for a project entitled, "*The alveolar epithelium: a target for viral infection and initiation of the immune response to viral infection."* The aims of this project are to 1) identify the cellular receptors that initiate expression of interleukin-1 in response to murine coronavirus infection and 2) identify the cellular proteins that are needed to process and release interleukin-1 from the infected cell. I am also a co-PI on a second seed project from the UI COBRE, entitled, "*Identify T. gondii proteins that inhibit airway inflammation in as asthma model."* In this project, we will use the protozoan parasite, *Toxoplasma gondii*, and proteins from the parasite to inhibit allergic inflammation in mice as a potential therapy for asthma. Neither of these projects have scientific overlap with the proposed research, and both will be completed by August 31, 2012, within two months of this Seed Grant project.

BIBLIOGRAPHY

1. Martin, E.T., et al., *Multiple versus single virus respiratory infections: viral load and clinical disease severity in hospitalized children.* Influenza Other Respi Viruses, 2012. **6**(1): p. 71-77.
2. Paranhos-Baccala, G., et al., *Mixed respiratory virus infections.* J Clin Virol, 2008. **43**(4): p. 407-10.
3. Aberle, J.H., et al., *Single versus dual respiratory virus infections in hospitalized infants: impact on clinical course of disease and interferon-gamma response.* Pediatr Infect Dis J, 2005. **24**(7): p. 605-10.
4. Johnson, J.E., et al., *The histopathology of fatal untreated human respiratory syncytial virus infection.* Mod Pathol, 2007. **20**(1): p. 108-19.
5. Wark, P.A., et al., *Diversity in the bronchial epithelial cell response to infection with different rhinovirus strains.* Respirology, 2009. **14**(2): p. 180-6.
6. Mosser, A.G., et al., *Similar frequency of rhinovirus-infectible cells in upper and lower airway epithelium.* J Infect Dis, 2002. **185**(6): p. 734-43.
7. Sanders, C.J., P.C. Doherty, and P.G. Thomas, *Respiratory epithelial cells in innate immunity to influenza virus infection.* Cell Tissue Res, 2011. **343**(1): p. 13-21.
8. Miura, T.A. and K.V. Holmes, *Host-pathogen interactions during coronavirus infection of primary alveolar epithelial cells.* J Leukoc Biol, 2009. **86**(5): p. 1145-51.
9. Wagner, A.M., J.K. Loganbill, and D.G. Besselsen, *Detection of sendai virus and pneumonia virus of mice by use of fluorogenic nuclease reverse transcriptase polymerase chain reaction analysis.* Comp Med, 2003. **53**(2): p. 173-7.
10. Slater, L., et al., *Co-ordinated role of TLR3, RIG-I and MDA5 in the innate response to rhinovirus in bronchial epithelium.* PLoS Pathog, 2010. **6**(11): p. e1001178.
11. Vester, D., et al., *Real-time RT-qPCR assay for the analysis of human influenza A virus transcription and replication dynamics.* J Virol Methods, 2010. **168**(1-2): p. 63-71.
12. Miura, T.A., et al., *Rat coronavirus infection of primary rat alveolar epithelial cells.* Adv Exp Med Biol, 2006. **581**: p. 351-6.
13. Lee, Y.M., et al., *Dual mechanisms of pestiviral superinfection exclusion at entry and RNA replication.* J Virol, 2005. **79**(6): p. 3231-42.
14. Zou, G., et al., *Exclusion of West Nile virus superinfection through RNA replication.* J Virol, 2009. **83**(22): p. 11765-76.
15. Wu, W., et al., *Characterization of the interaction between human respiratory syncytial virus and the cell cycle in continuous cell culture and primary human airway epithelial cells.* J Virol, 2011. **85**(19): p. 10300-9.
16. Garcia-Sastre, A., *Inhibition of interferon-mediated antiviral responses by influenza A viruses and other negative-strand RNA viruses.* Virology, 2001. **279**(2): p. 375-84.
17. Gotoh, B., et al., *Paramyxovirus strategies for evading the interferon response.* Rev Med Virol, 2002. **12**(6): p. 337-57.
18. Baig, E. and E.N. Fish, *Distinct signature type I interferon responses are determined by the infecting virus and the target cell.* Antivir Ther, 2008. **13**(3): p. 409-22.